# AN ELECTRON MICROSCOPE STUDY OF PROTOZOAN FLAGELLA

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## Introduction

While the magnifications obtained by the electron microscope have added greatly to the knowledge of the morphology of bacteria, the protozoa have been found to be too thick for easy penetration (unpublished work). However, it was believed that certain protozoan structures could be studied to advantage, and the following paper summarizes some results of electron microscope studies of flagella.

Flagella have been studied extensively with the light microscope. Vlk (1939) demonstrated several types among which was the ciliary type consisting of a shaft on which were located numerous small cilia, or very fine fibrils. Other biological fibers have been studied and are being investigated by means of the electron microscope. Jakus (1945) studied the morphology of trichocysts of *paramecia* elucidating the "ultra-structure" of the shaft. The shaft material is made of fibers which show long spacings typical of contractile fibers. These spacings, which probably represent the fundamental molecular arrangement in the fiber, differ slightly in length from the spacings in myosin and collagen. The connective tissue fiber, collagen, has been studied by Schmitt, Hall, and Jakus (1942) using the electron microscope. They were able to measure directly the long spacings which had previously been studied by Clark, Parker, Schaad, and Warren using X-ray diffraction techniques (1935), and had been remeasured by Bear (1942, 1944). Hall, Jakus, and Schmitt (1945) demonstrated several distinct long spacings in muscle fibers.

Fibers have been observed by electron micrograph in the tails of sperm by Baylor, Nalbandov, and Clark (1943) using bull sperm, and by Harvey and Anderson (1943) using the sperm of the sea urchin. The axial filament of the sperm tail in unfixed preparations separated into about ten to fifteen fibrils either in the region of the naked filament or in the region of a break in the protoplasmic sheath. The flagella of bacteria have been studied by Mudd, Polvitsky, and Anderson (1942) using the electron microscope.

By all odds the outstanding investigation on flagella, both in structure and function, is that of Harley P. Brown (1945). This Ph.D. thesis gives an exhaustive historical background as well as an account of the author's extensive experiments ranging from electron micrographs to underwater swimming. This paper unfortunately did not come to the attention of the present authors until a considerable amount of our work had been completed. Actually, there is very little to be added to the work of Brown. But it seems of value to record our independent observations, most of which are on quite different organisms. Our electron micrographs in part confirm the data of Brown, but those included in this paper primarily present new information on flagella not disclosed in his electron micrographs.

Brown studied the following organisms: *Astasia klebsii* Lemmermann, *Euglena gracilis* Klebs, *Ochromonas variabilis* Meyer, and *Chilomonas paramecium* Ehrenberg. The micrographs show that the flagella are of approximately uniform diameter throughout their entire length; that each flagellum consists of a denser axial core (axoneme) and a less dense sheath surrounding the core; that in the flagella of *Euglena* and *Astasia* the axial core appears to consist of two fibers of equal size; that the sheath appears to contain or consist of a coiled fiber in a helix encircling the axial core; that flagella of *Euglena* and *Astasia* bear along one side a single row of delicate filaments, designated flimmer, while the long flagellum of *Ochromonas* bears similar flimmer along both sides, and that of *Chilomonas* bears none. The general conclusion of Brown is that the flagellum beats in spiral undulations, confirming the theory of Lowndes (1945).

## Materials and Methods

The R.C.A. electron microscope, type B, was used in the following experiments. The microscope and its application to biology has been described in detail by Marton (1941), and by Zworykin, Marton, Ramberg, Hillier and Vance (1945). The specimen was placed directly on a collodion membrane supported by a standard wire mesh which served as the "slide." In the experiments which follow it was often desirable to examine the specimens at low magnifications so as to correlate the results with those of the light microscope. It was also desirable to view a wide field. The wide field, low magnification and greater depth of focus were obtained by placing the "slide" in the top of the specimen holder. The optical principles involved have been described by Burton, Barnes, and Rochow (1942). The technique of calibration described in that paper was employed to obtain the magnification figures used in this work.

Mixed cultures of protozoa were obtained from water from the Boneyard Creek in Urbana, Illinois. They were subcultured in chlorine-free water to which a grain of unpolished rice was added. Some pure cultures were attempted by subculturing from single organisms picked from dilute mixed cultures by fine pipette, but results of this procedure were unsatisfactory both as to photographs obtained and purity of culture. Saline suspensions of the gut contents of the cockroach *Peraplanita americana* were prepared in an attempt to photograph the flagella of the flagellates *Lophomanas blattarum* and *L. striata*. In a similar manner, suspensions of the gut contents of the termite *Reticulitermes flavipes* were prepared in order to observe the flagella of this rich flagellate fauna.

Specimens were prepared in the following ways:

1. A drop of culture was placed directly on the collodion membrane and allowed to dry in air. Some of these were washed in distilled water to remove excess debris, organisms, or salt.

2. A drop of the culture was placed directly on the collodion membrane, allowed to stand five to ten minutes in air, and then before complete drying had occurred, the screen was touched to a meniscus of distilled water. The surface tension of the meniscus drew the liquid from the screen leaving the biological material attached to the membrane.

3. Organisms were fixed in formalin directly on the screens, which were then washed in the above manner to remove the excess formalin.

4. Mixed cultures were fixed and stained with osmium tetroxide by adding 2 per cent $OsO_4$ in aqueous solution to the drop containing the organisms, allowing the organisms to sink, and then pipetting them to the screen.

## Results

Most of the protozoa were too large and thick for penetration by the electron beam. Specimens of *Euglena* sp. allowed no penetration, either in the region of the cell body or through the flagellum itself. Preparations from the gut of both cockroach and termite contained sufficient extraneous matter to obscure the structural details of the locomotor organelles of the flagellates. However, flagellates from the fresh-water cultures, some of which were tentatively assigned to the genus *Monas*, did allow enough penetration after drying to permit the cell outline and granules within the cell to be observed. Since this favorable material possessed several different and distinct types of flagellar forms, it was used for most of the studies herein described.

The general cell form of the organisms studied can be observed from the electron micrographs. Figure 1 shows a round cell body of *Monas* sp. which has undoubtedly flattened and shrunk during drying. The cell membrane is clearly seen, as is the protoplasmic mass which has become distorted during drying. Numerous granules can be seen within the cell, but their identification can only be surmised. The unidentified flagellate in Figure 2 shows an invagination at the apex behind which is a clear area, suggesting a cytostome and reservoir, or perhaps a contractile vacuole.

The fixation techniques employed in this work were not of a nature to demonstrate delicate cellular or flagellar components. Denaturation of protein appears to render it more opaque to the electron beam as does certainly the addition of a heavy metal. Formalin fixation caused adherence of foreign material to the cell body. The effects of osmium tetroxide can be seen in Figures 4, 6, 7, and 10. Much debris is always adsorbed and the cell body is completely impenetrable. The freezing-drying technique (Wykoff, 1946) is suggested as being probably the best to preserve the form of these animals for study with the electron microscope.

The flagella seen in the accompanying photographs are of two types, the ciliary flagellum and the fibrous flagellum. Vlk (1938) named the ciliary flagellum and found it present in many species of flagellates. He called the type without cilia the whip-flagellum, which may be the same as the type observed in this work and designated as the fibrous flagellum owing to the nature of its structure. The ciliary type seen in Figures 1 through 7 appears to consist of a shaft or tube, or mass of densely packed elongate fibers surrounded by radially placed ciliary structures (or flimmer). The exact nature of the central shaft cannot be determined from the present photographs. At the distal end of the flagellum shown in Figure 1, and in the region of the bend in Figure 5 long heavy fibers may be seen. The shaft may be a tube of these, or may have them embedded in the tube wall as a contractile element. In Figures 2 and 3 the shaft appears solid. In Figure 7 the central structure, which has been fixed with osmic acid, may possibly be interpreted as a hollow tube. It is probable that there are several different sub-types of the ciliary type of flagellum. In numerous cases one of the two flagella measured less than one micron in length. Flagella usually measured from 5 to 10 microns in length, and about 0.3 microns in width.

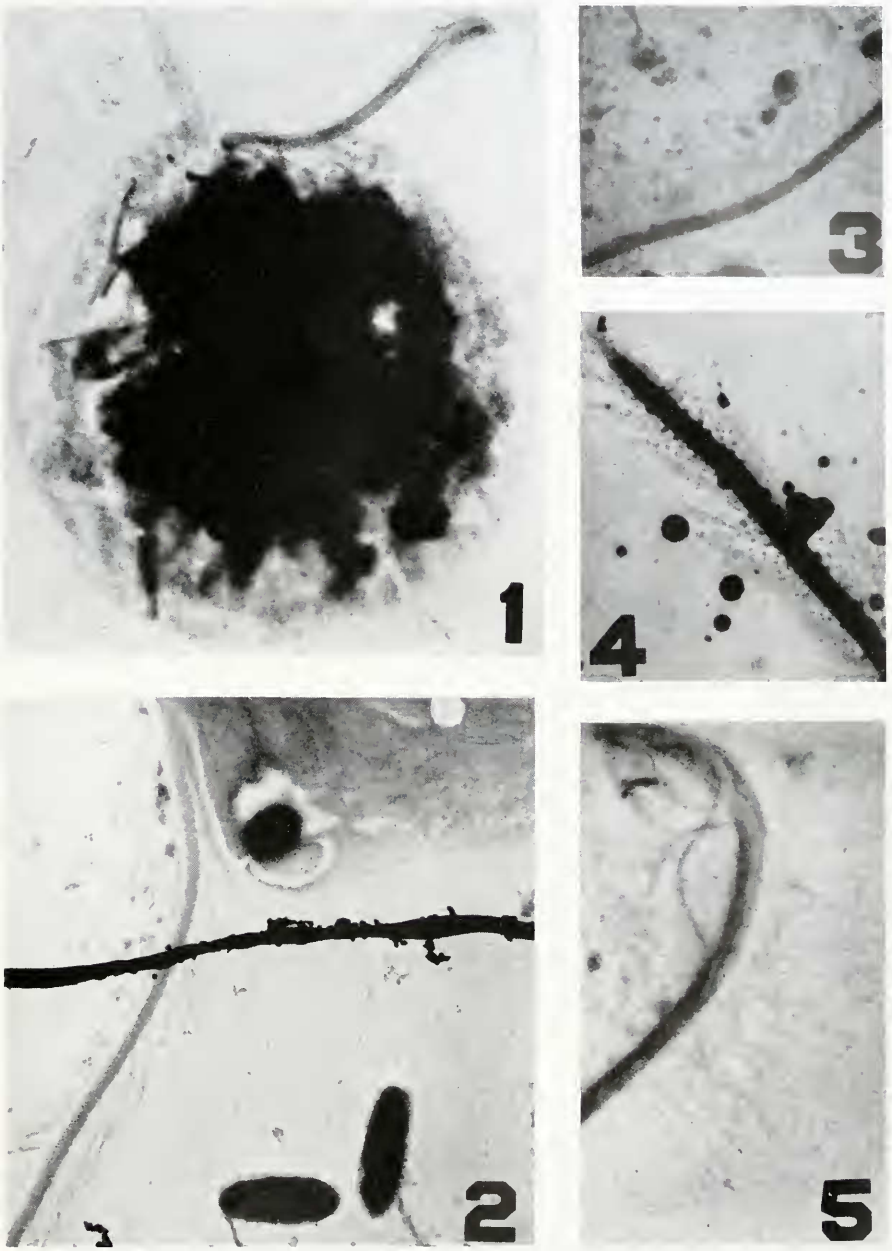


PLATE I

FIGURE 1. Electron micrograph of "*Monas* sp." showing cell body and attachment of two types of flagella (ca. 5,700 ×).

FIGURE 2. Organism (unidentified) showing single ciliary flagellum. Note shaft nodules and arrangement of cilia (ca. 4,700 ×).

FIGURE 3. Portion of ciliary flagellum showing nodules of shaft and cilia (ca. 6,600 ×).

FIGURE 4. Ciliary flagellum after osmic acid fixation (ca. 8,400 ×).

FIGURE 5. Ciliary flagellum with fibrous shaft (ca. 9,600 ×).

The point of attachment of the cilia on the flagella could not be determined by these photographs. Figures 2 and 3 show small nodules limited to one side of the shaft from which groups of cilia appear to take their origin. These are most dense in the proximal region. Brown ascribed this appearance to the helical fibrous sheath closely appressed to the axial core. Figures 1 and 5 show no such nodules although the cilia are clearly seen. In Figure 4 the form of the shaft is obscured by clumps of osmic acid which have also deposited between the individual cilia.

The fibrous flagella are best seen in Figures 8 and 9. They appear to be long fibers twisted spirally as the fibers of a rope. The rope-like quality is especially clear in Figure 9 where the tip of the flagellum has frayed. This structure was not observed in the microscope of Brown. Figure 8 shows the base of a flagellum which has broken away from the rest of the cell. These were not uncommon. The fibers appear to extend into the circular portion which is probably a part of the cell body. It would be interesting to observe isolated portions of the cell membrane for long fibers.

Long spacings were sought in the fibers. Enlargement of the fiber showed none. However, only very excellent photographs at high magnifications and at perfect focus can be used for these determinations. In osmic acid fixed rat tail collagen we were able to observe the long spacings reported by Schmitt, Hall and Jakus (1942). Hall, Jakus and Schmitt (1945) were able to demonstrate spacings in myosin only after treatment with either osmic acid or phosphotungstic acid. Figure 10 is a high magnification of the flagellar fibers treated with osmic acid. The granulation seen in the fibers is very regular, and paired rows of granules appear to lie together. This is the only indication of a periodicity which has been found in this work. It may be an artifact caused by precipitated osmic acid enmeshed in the fibrils. Very careful additional work should be carried out in this direction.

## Discussion

The agreement of the gross morphology of protozoans as revealed by electron micrographs with that seen by light microscope studies suggests that additional information may be gained by the electron microscope. The danger of artifacts introduced by the necessary treatment for such observations can be overcome by careful studies of morphological changes under different treatments and can be at least reduced to the level of that found in treatment preliminary to light microscope examination. The use of stains, in some cases specific, which are suitable to the optics of the electron system has been attempted, and will no doubt be developed to a satisfactory state. (Baylor, Appleman, Sears and Clark (1945) state that the electron "stain" must differentially alter the densities of various protoplasmic constituents.) The present day viewpoint of the necessity of knowledge of the specificity of a stain, and its action on well defined chemical constituents, ought to preserve the new field of electron microscopy from the array of sometimes meaningless colors now burdening light microscopy.

Needless to say, there is real need for careful identification of the specimens examined. Such was not possible at the time of this work. The necessity for pure cultures in investigations such as these is evident.

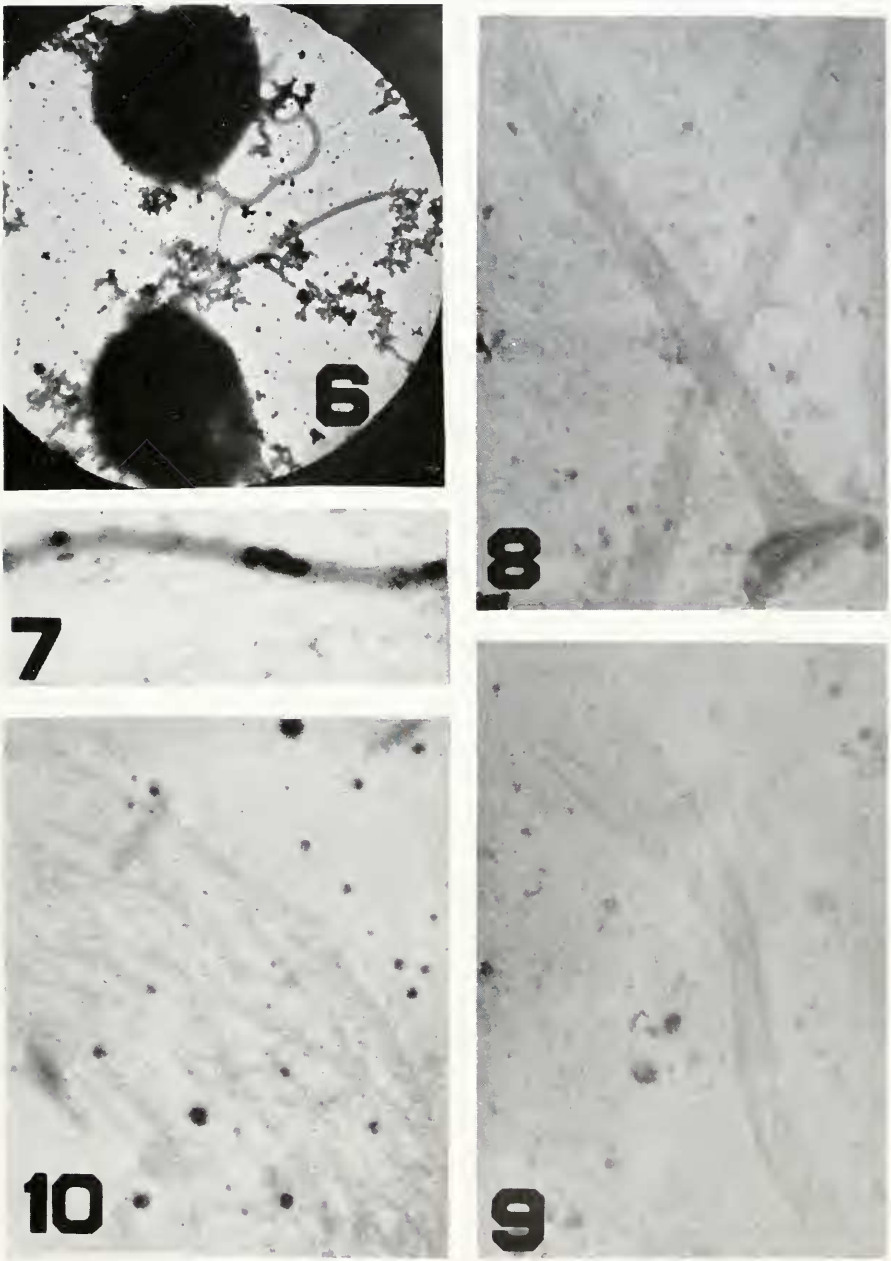


PLATE II

FIGURE 6. Two unidentified flagellates after osmic acid fixation (ca. 4,500 ×).
FIGURE 7. Osmic acid fixed ciliary flagellum, the shaft appears hollow (ca. 17,000 ×).
FIGURE 8. Fibrous flagellum broken away from the cell body (ca. 7,500 ×).
FIGURE 9. Fibrous flagellum showing rope-like arrangement of fibers (ca. 7,500 ×).
FIGURE 10. High magnification of frayed fibrous flagellum after osmic acid fixation (15,000 ×).

A mechanism of flagellar movement is suggested by the electron micrographs. The presence of fibrils in both types of flagella suggests that contraction and relaxation occurs as it is believed to occur in the fibers of muscle and connective tissue, that is, by molecular rearrangement. Contraction of individual fibers, particularly those arranged in the rope-like fashion seen in Figure 9, could account for the sweeping spiral movements observed in the living state. There is an indication that the fibrils of the fibrous type may extend into the cell body. Unilateral contraction of those in the cell body could certainly account for a variety of movements. The cilia of the ciliary type may function in either the original sweep or the recovery. They may also function in creating favorable currents in the water environment. In Figure 5 the cilia appear to be an extension of the protoplasmic membrane, and since no actual fibers can be observed within them, it may be that they serve to increase the sensory, absorptive or secretory surface of the cell.

The fibrous constitution of certain of the flagella indicates a broad distribution of fibers throughout the animal kingdom, from these simple organisms up through the complexities of the highly developed mammals. Are the fiber types the same throughout the whole kingdom? Is collagen, for example, present in all living animal forms, or does some other fiber take over its function in other species? Does the fiber form determine the function? Solution of these problems awaits further investigation and development of new and improved techniques.

## Summary

Certain flagellated protozoa have been photographed by the electron microscope. Two well defined types of flagella have been observed, a fibrous type in which the individual fibers occur in a twisted rope-like arrangement, and a ciliary type on which are arranged numerous small cilia. The shaft of the ciliary type in some cases appears to consist of fibrils. These observations verify in part and augment the masterly work of Harley P. Brown at The Ohio State University.

## LITERATURE CITED

Baylor, M. R. B., A. V. Nalbandov, and G. L. Clark, 1943. Electron microscope study of sperm. *Proc. Soc. Exp. Biol. Med.*, **54**: 229–232.

Baylor, Martha B., M. D. Appleman, O. H. Sears, and G. L. Clark, 1945. Some morphological characteristics of nodule bacteria as shown by the electron microscope. II. *Jour. Bact.*, **50**: 249–256.

Bear, R. S., 1942. Long X-ray diffraction spacings of collagen. *Jour. Am. Chem. Soc.*, **64**: 727.

Bear, R. S., 1944. X-ray diffraction studies on protein fibers. I. The large fiber-axis period of collagen. *Jour. Am. Chem. Soc.*, **66**: 1297–1305.

Brown, Harley P., 1945. On the structure and mechanics of the protozoan flagellum. *Ohio Jour. Sci.*, **45**: 247–301.

Burton, C. J., R. B. Barnes, and T. G. Rochow, 1942. The electron microscope, calibration and use at low magnifications. *Ind. Eng. Chem.*, **34**: 1429–1436.

Clark, G. L., E. A. Parker, J. A. Schaad, and W. J. Warren, 1935. New measurements of previously unknown large interplanar spacings in natural materials. *Jour. Am. Chem. Soc.*, **57**: 1509.

Hall, C. E., M. A. Jakus, and F. O. Schmitt, 1942. Electron microscope observations of collagen. *Jour. Am. Chem. Soc.*, **64**: 1234.

Hall, C. E., M. A. Jakus, and F. O. Schmitt, 1945. The structure of certain muscle fibrils as revealed by the use of electron stains. *Jour. Applied Phys.*, **16**: 459–465.

HARVEY, E. B., AND T. F. ANDERSON, 1943. The spermatozoon and fertilization membrane of Arbacia punctulata as shown by the electron microscope. *Biol. Bull.*, **85**: 151–156.

JAKUS, MARIE A., 1945. The structure and properties of the trichosysts of paramecium. *Jour. Exp. Zool.*, **100**: 457–486.

LOWNDES, A. G., 1945. Swimming of Monas stigmatica. *Nature*, **155**: 579.

MARTON, L., 1941. The electron microscope, a new tool for bacteriological research. *Jour. Bact.*, **41**: 397–413.

MUDD, S., KATHERINE POLEVITZKY, AND T. F. ANDERSON, 1942. Bacterial morphology as shown by the electron microscope. IV. Structural differentiation within the bacterial protoplasm. *Arch. Path.*, **34**: 199–207.

SCHMITT, F. E., C. E. HALL, AND MARIE JAKUS, 1942. Electron microscope investigations of the structure of collagen. *Jour. Cell. Comp. Physiol.*, **20**: 11–33.

VLK, VLADIMIR, 1938. Über den Bau der Geissel. *Arch. f. Protistenk.*, **90**: 448–488.

WYKOFF, R. W. G., 1946. Frozen dried preparations for the electron microscope. *Science*, **104**: 36–37.

ZWORYKIN, V. K., G. A. MORTON, E. G. RAMBERG, J. HILLIER, AND A. W. VANCE, 1945. *Electron optics and the electron microscope*. John Wiley and Sons, Inc. New York.